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TITLE: Assessment of Biomarkers Associated with Joint Injury and Subsequent Post-Traumatic Arthritis

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14. ABSTRACT The overall objective of this research effort is to identify biomarkers following articular fracture that may be predictive of the development of post-traumatic arthritis (PTA). PTA is a clinically important complication of joint injury with life-long effects for the patient. While PTA can occur rapidly after moderate to severe articular injuries, not every patient will go on to develop this condition. There are no effective screening methods to determine who is at risk. This proposal includes both a clinical observational study and a series of murine experiments, both with the goal of identifying biomarkers that are associated with development of PTA. Patients with knee joint fractures will be enrolled, and we will collect serum, urine, and synovial fluid early after injury. Radiographic imaging will be performed early after injury, again at 18 months, and analyzed to determine which patients developed PTA from those who did not. We will assess the ability of identified biomarkers in serum, urine, and synovial fluid to predict PTA following joint injury. Additionally, biomarkers will be assessed in a murine model of articular fracture using two strains in which one strain develops PTA and the other does not. Comparison of the human and mouse response to knee joint fracture will allow assessment of the potential use of the mouse model to evaluate future therapies to prevent PTA.					
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"Assessment of Biomarkers Associated with Joint Injury and Subsequent Post-Traumatic Arthritis"  
Start date: 9/30/2012  
PIs – Steven A. Olson (SAO); Farshid Guilak (FG); and Virginia B Kraus (VBK)

## **1. INTRODUCTION:**

Post-traumatic arthritis (PTA) is a clinically important complication of joint injury with life-long effects for the patient<sup>1,2</sup>. PTA is a severe burden in active duty and discharged soldiers<sup>3</sup>. While PTA can occur rapidly after moderate to severe articular injuries, not every patient will go on to develop this condition. There are no effective screening methods to determine who is at risk for developing PTA. The overall objective of this proposal is to identify biomarkers following articular fracture that may be predictive of the development of PTA. To accomplish this we will conduct a two-part study. We will perform a prospective observational study of patients with lower extremity articular fractures requiring operative treatment. Patients with knee joint fractures will be enrolled, and we will collect serum, urine, and synovial fluid from each patient acutely after injury. Radiographic imaging will be performed early after injury and again at 18 months. Both scans will be analyzed to separate the patients that developed PTA from those who did not. We will assess the ability of identified biomarkers in serum, urine, and synovial fluid to predict PTA following joint injury. Additionally, biomarkers will be assessed in a murine model of articular fracture using two strains in which one strain develops PTA and the other does not. Comparison of the human and mouse response to knee joint fracture will allow for assessment of the potential use of the mouse model to evaluate future therapies to prevent PTA. The low cost of mouse models lends itself to this type of work, and the results will provide a validated model to use for studying PTA. The goal of this work is to establish the basis for future use of biomarkers to predict the potential risk for developing PTA after acute joint injury. In addition this work will elucidate data on biospecimens that may be useful in future registries of acute joint injuries.

## **2. KEYWORDS:**

Post-traumatic arthritis, post-traumatic osteoarthritis, articular fracture, joint injury, trauma, biomarker, inflammation, MRI, knee, mouse model, translational research.

## **3. OVERALL PROJECT SUMMARY:**

### **Section 3 – Overall Progress to date**

Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion.

The overall objective of this study is to identify biomarkers following articular fracture that may be predictive of the development of PTA. Specifically, patients with a closed unilateral articular fracture of the knee requiring operative treatment will be enrolled over an 18-month period. Biosamples (synovial fluid from the injured and contralateral uninjured knee, serum, and urine) will be collected prior to or at surgical intervention. MRI imaging of the injured knee will be obtained to assess the articular cartilage. Degenerative changes in the cartilage and joint space narrowing will be correlated to biomarkers that may be indicative and predictive of joint degeneration and the development of PTA. We have successfully enrolled patients, collected and stored biosamples, and have begun receiving MRI scans for study patients (Figure 1). Enrollment was initially slow. However, we addressed this issue by expanding the enrollment criteria. Sample collection and processing has been very successful, and we are pleased with the quantity of biosamples collected from each patient.

A second aim of this study is to create closed tibial plateau fractures in the left knee of C57BL/6 mice that develop PTA and MRL/MpJ mice that are protected from PTA. Serum and synovial

fluid will be collected from both strains at various time points. Biospecimens will be analyzed for markers of joint inflammation and degradation identified in the human knee following articular injury. Biomarkers will be correlated to joint pathology that will be assessed from microCT and histology. The human and mouse biomarker profiles associated with PTA will be compared to assess correlations between them. We have successfully completed the short-term data collection (pre-fracture, 0, 1, 7 and 14 days post-fracture), including receiving animals, fracturing, sacrificing, and collection of biosamples. Hind limbs have been scanned with microCT (Figure 2) and processed for histology. We will be working on the data analysis during the second year. For the long-term animal portion of the study (8 weeks post-fracture), we have received the animals on site and will complete the fracturing, sacrificing, and collection of biosamples for the mice. Data analysis will begin during the second year for some portions of the long-term mice. Minor issues with the supplier for the animal portion of the grant have been addressed and we anticipate no issues in the future.

The details of our progress to date are described below with each task outlined in the approved Statement of Work (SOW).

***Timeline:*** description of progress for each task listed in bullet points

#### Specific Aim 1

**Task 1.** Review and approval of IRB protocol (months 1-4) [SAO, VBK]

- Duke IRB application submitted on 05/31/2012
- Duke IRB application approved on 07/11/2012
- Amendment to Duke IRB protocol to expand enrollment criteria submitted on 05/06/2013
- Amendment to Duke IRB protocol to expand enrollment criteria approved on 05/17/2013

**Task 2.** USAMRMC Office of Research Protections review and approval of human use documents (months 1-6) [SAO]

- IRB application submitted to USAMRMC Office of Research Protections for review on 09/06/2012
- IRB application approved by USAMRMC Office of Research Protections on 09/21/2012
- Request to expand enrollment criteria was submitted with prior progress report on 04/09/2013
- Response from USAMRMC ORP HRPO received on 05/02/2013.
  - We were informed that the expanded enrollment criteria was not a substantive modification/amendment to our protocol and does not increase risk to subjects. Therefore, the only action needed was to amend the protocol and submit to our Duke IRB for expedited review/approval.

**Task 3.** Enroll 30 patients in study (months 4-18) [SAO, FG, VBK]

3a. Patients with closed unilateral articular fracture of the knee requiring operative treatment will be enrolled in study

3b. Biosamples (synovial fluid, blood, urine) will be collected at time of placing a temporizing spanning external fixator

- First patient enrolled on 12/19/2012 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Second patient enrolled on 03/06/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Third patient enrolled on 06/19/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Fourth patient enrolled on 07/03/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Fifth patient enrolled on 07/18/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Sixth patient enrolled on 07/23/2013 [SAO]

- Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Seventh patient enrolled on 07/25/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Eighth patient enrolled on 08/23/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]
- Ninth patient enrolled on 09/12/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG, VBK]

*Task 4.* Visual analog pain score, the Knee injury and Osteoarthritis Outcome Score (KOOS), and the SF-36 will be completed within 2 weeks of injury.

- Visual analog pain score, KOOS, and SF-36 for first patient was completed on 12/27/2012 [SAO]
- Visual analog pain score, KOOS, and SF-36 for second patient was completed on 03/06/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for third patient was completed on 06/20/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for fourth patient was completed on 07/01/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for fifth patient was completed on 07/17/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for sixth patient was completed on 07/22/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for seventh patient was completed on 07/23/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for eighth patient was completed on 08/23/2013 [SAO]
- Visual analog pain score, KOOS, and SF-36 for ninth patient was completed on 09/13/2013 [SAO]

*Task 5.* Biosamples (synovial fluid, blood, urine) will be collected at time of definitive fixation of closed unilateral articular fracture of the knee requiring operative treatment. Timing of repair will be based on standard of care for treating the clinical injury.

- Biosamples from first patient collected at time of definitive fixation on 12/22/2012 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG]
- Biosamples from second patient collected at time of definitive fixation on 03/13/2013 [SAO]
  - Synovial fluid, blood, urine processed and stored in -80° freezer [FG]
- The third patient enrolled had definitive fixation at the baseline visit on 06/19/2013 so a second set of biosamples was not collected.
- The fourth patient enrolled had definitive fixation at the baseline visit on 07/03/2013 so a second set of biosamples was not collected.
- The fifth patient enrolled had definitive fixation at the baseline visit on 07/18/2013 so a second set of biosamples was not collected.
- The sixth patient enrolled had definitive fixation at the baseline visit on 07/23/2013 so a second set of biosamples was not collected.
- The seventh patient enrolled had definitive fixation at the baseline visit on 07/25/2013 so a second set of biosamples was not collected.
- The eighth patient enrolled had definitive fixation at the baseline visit on 08/23/2013 so a second set of biosamples was not collected.
- The ninth patient enrolled had definitive fixation at the baseline visit on 09/12/2013 so a second set of biosamples was not collected.

**Task 6.** Post-operative follow-up of all patients (months 5-18) [SAO]

6a. Post-operative T1-rho MRI will be obtained

6b. Analysis of MRI T1-rho imaging of cartilage

- Post-operative MRI obtained from first patient on 01/30/2013 [SAO]
- Post-operative MRI obtained for second patient on 04/30/2013 [SAO]
- Post-operative MRI attempted for third patient on 08/06/2013; patient experienced claustrophobia associated with the MRI machine and may not return to study [SAO]
- Analysis of MRI for first and second patients has been begun. [SAO]
- Post-operative MRI not obtained for fourth patient because patient will have a total knee replacement; patient does not satisfy enrollment criteria and has been removed from the study [SAO]
- Post-operative MRI is in the process of being scheduled for fifth patient [SAO]
- Post-operative MRI obtained for sixth patient on 09/23/2013 [SAO]
- Post-operative MRI obtained for seventh patient on 09/26/2013 [SAO]
- Post-operative MRI not obtained for eighth patient because definitive fixation resulted in just an external fixation; patient does not satisfy enrollment criteria and has been removed from the study [SAO]
- Post-operative MRI scheduled for ninth patient the week of 10/28/2013 [SAO]

**Supporting Data**

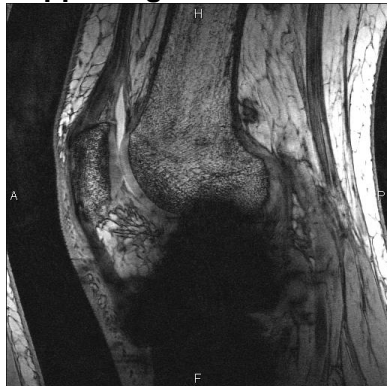


Figure 1: MRI slice from study patient with a tibial plateau fracture.

**Specific Aim 2**

**Task 1.** Review and approval of animal protocol (months 1-4) [FG]

- Duke IACUC application submitted on 07/02/2012
- Duke IACUC application approved on 07/25/2012
- Duke certification of IACUC Review and Approval/Grant concordance received on 08/21/2012
- Amendment to Duke IACUC to allow use of live microCT scanning submitted 06/10/2013.
- Amendment to Duke IACUC to allow use of live microCT scanning approved 07/17/2013.

**Task 2.** USAMRMC Office of Research Protections review and approval of animal use documents (months 1-4) [FG]

- ACURO animal use appendix submitted to USAMRMC Office of Research Protections for review on 08/29/2012
- Received ACURO approval letter on 11/29/2012
- Amendment to allow use of live microCT scanning submitted to ACURO 08/12/2013.
- Amendment to allow use of live microCT scanning approved to ACURO 08/20/2013.

**Task 3.** Obtain mice and create closed intra-articular fracture of the left knee of mice (months 5-9) [FG]



- 3a. Obtain C57BL/6 and MRL/MpJ mice at 8 weeks of age
  - 60 mice were ordered on 01/07/2013 [FG]
    - 30 C57BL/6 mice were received at 9 weeks of age on 01/16/2013 [FG]
    - 30 MRL/MpJ mice were received at 10 weeks of age on 01/24/2013 [FG]
  - Replacement C57BL/6 mice were ordered after receiving credit for 12 mice on 05/13/2013 [FG]
    - 12 C57BL/6 mice were received at 10 weeks of age on 05/22/2013 [FG]
    - 2 C57BL/6 mice died due to unidentified health reasons on 05/26/2013 [FG]
    - 2 replacement C57BL/6 mice were received after credit on 08/07/2013 [FG]
- 3b. Allow mice to mature to 16 weeks of age
  - Mice (30 MRL/MpJ and 18 C57BL/6) were housed until 03/04/2013 [FG]
  - 10 C57BL/6 mice were housed until 07/17/2013 [FG]
- 3c. Create closed intra-articular fractures in the left knee of mice
  - Fractures were created 03/05/2013 – 03/13/2013 [FG]
  - Fractures (n=6) were created on 07/17/2013 [FG]

*Task 4. Sacrifice mice and harvest samples for analyses (months 10-11) [FG]*

- 4a. Sacrifice mice at pre-fracture, 0, 1, 7 and 14 days
- 4b. Collect serum and synovial fluid at time of sacrifice and store in -80° freezer fracture
- 4c. Harvest both hind limbs for analyses, store in -20° freezer
  - Mice were sacrificed (MRL/MpJ at pre-fracture, 0, 1, 7 and 14 days post-fracture and C57BL/6 at 0,1, and 7 days post-fracture) on 03/04/2013 – 03/27/2013 [FG]
    - Serum and synovial fluid were collected at time of sacrifice and stored in -80° freezer [FG, VBK]
    - Both hind limbs were harvested at time of sacrifice and stored in -20° freezer [FG, VBK]
  - Mice were sacrificed (C57BL/6 at pre-fracture & 14 days post-fracture) on 07/31/2013 [FG]
    - Serum and synovial fluid were collected at time of sacrifice and stored in -80° freezer [FG, VBK]
    - Both hind limbs were harvested at time of sacrifice and stored in -20° freezer [FG, VBK]
  - Mice were sacrificed (C57BL/6 at pre-fracture) on 08/08/2013. [FG]
    - Serum and synovial fluid were collected at time of sacrifice and stored in -80° freezer [FG, VBK]
    - Both hind limbs were harvested at time of sacrifice and stored in -20° freezer [FG, VBK]

*Task 5. Perform microCT analyses on hind limbs (months 12-18) [FG]*

- Limbs were scanned 09/04/2013-09/09/2013 [FG]
  - Data processing and analysis will begin in the next month [FG]

*Task 7. Obtain 24 additional mice and create closed intra-articular fracture of the left knee of mice (month 12-16) [FG]*

- 7a. Obtain 12 C57BL/6 mice and 12 MRL/MpJ mice at 8 weeks of age
- 7b. Allow mice to mature to 16 weeks of age
- 7c. Create closed intra-articular fractures in the left knee of mice
  - 24 mice were ordered on 07/29/2013 [FG]
    - 12 C57BL/6 mice were received at 9 weeks of age on 08/07/2013
    - 12 MRL/MpJ mice were received at 8 weeks of age on 08/15/2013
    - 2 C57BL/6 mice died due to unidentified health reasons on 08/13/2013 [FG]
    - 2 replacement C57BL/6 mice were received after credit on 08/28/2013 [FG]



## Supporting Data

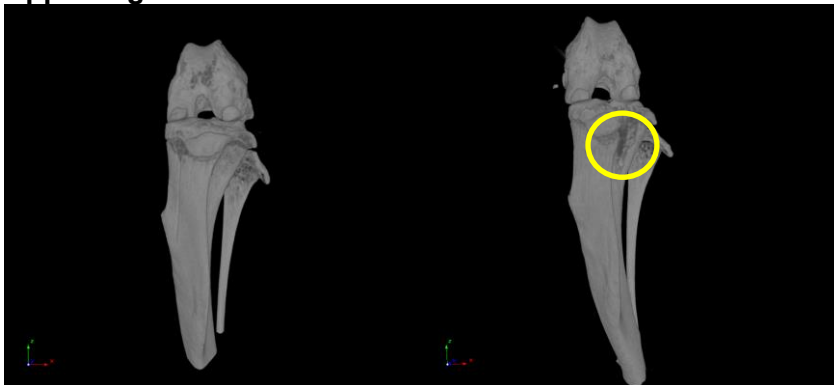


Figure 2: MicroCT image of a non-fracture (left) and fractured mouse knee (right). The fracture location is indicated by circle.

## Problem Areas

**Specific Aim 1:** We have noticed a slow patient enrollment over the first year of this study. In May, we expanded the enrollment criteria in order to increase the number of patients that would qualify for the study. The amendment to the IRB protocol added patients with a unilateral closed intra-articular fracture of the knee that required surgical treatment, but did not require a spanning external fixator. This resulted in the enrollment of 7 additional patients that would not have satisfied the original inclusion criteria. Additionally, three patients had to stop their participation in the study for the reasons described below. One patient experienced claustrophobia during the MRI scan and could not continue; one patient's treatment was converted to a total knee replacement; and one patient's definitive treatment was external fixation only with no ORIF. The biosamples obtained from these patients will be reserved. If enrollment continues at the current pace we may need a no cost extension to complete aim 1.

**Specific Aim 2:** We had issues with our animal supplier. The problem has been resolved and all credits have been issued, enabling us to maintain the same animal numbers that we proposed in the original grant.

## Animal Usage Stats

- DOD Annual Report on Animal Use:
  - Species used: mice
  - Number of each species used: 84
- USDA Pain Category for all animals used:
  - Category C (Non-Painful Procedures): 12
  - Category D (Procedures using anesthesia/analgesia): 72

## 4. KEY ACCOMPLISHMENTS:

- The protocol was approved for use of patients in this study and nine patients have been enrolled in the study.
- Biosamples of serum, plasma, synovial fluid and urine have been collected, processed and stored from patients enrolled.
- The sample collection and processing has been very successful. The relatively large volumes of samples collected will ensure that sufficient samples are available for the proposed biomarker analyses.
- The protocol was approved for use of animals in this study.

- Animals were obtained and closed articular fractures of the tibial plateau were successfully created with a 100% success rate.
- Animals were sacrificed at pre-fracture, 0, 1, 7 and 14 days post-fracture. Serum, synovial fluid and hind limbs were harvested.
- Hind limbs were scanned by microCT and processed for histological assessment.
- Additional mice have been ordered and received for the remaining time point of 8 weeks post-fracture. These mice will also be followed with in vivo microCT imaging.

## **5. CONCLUSIONS:**

Post-traumatic arthritis (PTA) is a severe burden in active duty and discharged soldiers. Recent figures from Operation Iraqi Freedom and Operation Enduring Freedom indicated joint degeneration following injury is the most common cause of a soldier being unfit for duty<sup>3</sup>. Compared to other forms of arthritis, (PTA) has a more rapid clinical onset<sup>4</sup>. This rapid onset of degenerative arthritis is occurring following joint injuries in a younger population of soldiers. The goal of this work is to identify biomarkers following articular fracture that may be predictive of the development of PTA. This knowledge is needed for future investigations to assess acute interventions to prevent PTA that can be given on the battlefield or at the time of stabilizing medical care in down range medical facilities. To reach this goal, the proposed investigation studies patients who have sustained a closed, displaced articular fracture about the knee requiring operative treatment. While these patients are not suffering battlefield injuries, they do represent significant articular injuries that are at risk for developing PTA. A companion murine bench top series of experiments will allow for the comparison of human and mouse response following joint fracture. An animal model is needed to allow for a low-cost model for the assessment of future therapies to prevent PTA.

**6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:** Nothing to report.

**7. INVENTIONS, PATENTS AND LICENSES:** Nothing to report.

**8. REPORTABLE OUTCOMES:** The existing measures for clinical outcome are being applied in a novel manner to articular fractures. Data points such as VAS pain scores, KOOS, and SF-36 have been completed by enrolled patients. The research is still in progress so reportable outcomes from the study are still pending.

## **9. OTHER ACHIEVEMENTS:**

Dr. Virginia Kraus has become a member of the executive committee to oversee the Arthritis Foundation ACL Consortium Project to develop and validate an MRI protocol for detecting ACL injury related pathology beginning within days of injury and extending to 6 months and beyond.

## **10. REFERENCES:**

1. CDC. *Arthritis: The Nation's Most Common Cause of Disability*. Chronic Disease At a Glance Reports - Arthritis 2011 February 18, 2011 [cited 2011 October 29]; Description of demographics of the burden of disease for arthritis in the United States.]. Available from: <http://www.cdc.gov/chronicdisease/resources/publications/aag/arthritis.htm#chart1>.
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3. Cross JD, Ficke JR, Hsu JR, Masini BD, Wenke JC. 2011. Battlefield orthopaedic injuries cause the majority of long-term disabilities. *The Journal of the American Academy of Orthopaedic Surgeons*. 19 Suppl 1:S1-7.

4. Furman BD, Olson SA, Guilak F. Nov-Dec 2006. The development of posttraumatic arthritis after articular fracture. J Orthop Trauma. 20(10):719-725.

**11. APPENDICES:** Nothing to report.